REMINERALIZING EFFECT OF DIFFERENT TOPICAL AGENTS ON OCCLUSAL SURFACES WITH OR WITHOUT CARIES: AN IN VITRO EVALUATION STUDY USING ELECTRICAL RESISTANCE MEASUREMENT

ABSTRACT

Background and Aim: To compare the remineralization effect of 3 caries preventive agents on occlusal surfaces with or without fissure caries.

Materials and Methods: Forty extracted partially erupted (with visible fissure caries-Group 1) and included (free of fissure caries-Group 2) human third molars were used. Occlusal surfaces of teeth were examined by Electronic Caries Monitor IV (ECM) and baseline values were obtained. In Groups 1 and 2, teeth were randomly assigned to 4 subgroups (n=10). A: Acidulated phosphate fluoride gel (APF); B: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); C: 5% NaF Varnish with amorphous calcium phosphate (NaF-ACP); D: (Control). After application of topical agents, teeth were subjected to pH cycling 14 days. ECM readings were repeated.

Results: In Group 1 and 2, all intra-group differences (except for 1D and 2D) were found significant (p<0.01 and p<0.05). For Group 1, all inter-group differences were significant (p<0.05). In Group 2, all subgroups significantly differed from 2D (control) (p<0.05) while, Group 2B was also significantly different from 2A and C (p<0.05). The differences for Group 1 and 2 were also significant (p<0.01).

Conclusion: All topical agents showed remineralizing effect on both carious and caries-free occlusal surfaces which were more pronounced on the latter surfaces. CPP-ACP significantly provided better results than APF and NaF-ACP on caries-free occlusal surfaces.

Key words: CPP-ACP, Electrical Resistance Measurement, Fluoride Varnish, Remineralization

Submitted for Publication: 05.23.2012
Accepted for Publication: 10.08.2012
INTRODUCTION

Tooth structure undergoes continuous demineralization and remineralization in the oral environment. When this balance is changed, demineralization will progress leading to a degradation of the tooth structure. Until recently, the conventional treatment concept for affected tooth was the removal of the affected tissues and restoration with a restorative material. Nowadays, contemporary approach is the non-invasive management of the caries lesions. It has been stated that if some therapeutic agents were applied to the teeth, non-cavitated lesions could be arrested. There is strong evidence that fluoride (F) has a beneficial effect on remineralization of the tooth structure. According to Clarkson, the reduction in dental caries prevalence observed during the last decades could be explained by the wide use of fluorides. Acidulated phosphate fluoride (APF) is an effective cariostatic agent that had been widely investigated in the last half century. In many countries, APF gels are frequently used in dental clinics and prevention programs in schools. Recently, a milk protein derivative, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) complex, has been introduced for caries prevention and enamel remineralization. Encouraging results have been reported with respect to the possible role of CPP-ACP in remineralization of enamel following erosion by acidic drinks; protection of dentine against acid erosion induced wear and in the control of cervical dentine hypersensitivity. CPP-ACP has been incorporated into oral health products such as chewing gum, mouth rinse and paste.

It has been known that fluoride varnishes were able to deposit large amounts of fluoride on enamel surfaces and have received significant attention for their potential in caries inhibition. These varnishes offer the advantages of slow fluoride release, ease of application and safety in the levels of released fluoride. In search of better diagnosis for occlusal caries, alternative methods have been investigated. Electrical resistance measurement is based upon the fact that caries enamel and dentine are more porous than the surrounding sound tissue and when filled with water and soluble electrolytes, (e.g. in presence of caries) the high electrical resistance of sound dental tissue decreases. With the use of electrical resistance measurement, this study aimed to compare the remineralization effect of caries preventive agents with different formulas on occlusal surfaces of teeth with or without fissure caries.

MATERIALS AND METHODS

After extraction, partially erupted and included human third molar teeth were collected into separate containers. Organic remnants on teeth were removed with a surgical blade and the occlusal surfaces of the teeth were cleaned with a bristle brush rotating on a slow-speed handpiece with irrigation. The teeth were then stored in 0.1% thymol solution (in separate containers) until the experimental procedures were initiated, thereby preventing dehydration. From this pool, teeth with visible fissure caries and without caries were allocated into groups, each having 40 teeth (Group 1 and 2, respectively). This grouping was also verified by the use of electrical resistance measurement (Electronic Caries MonitorType IV (ECM), Lode Diagnostics, The Netherlands). According to the severity scale provided, teeth with readings > 1 GigaOhm (GW) - 10.00 MegaOhm (Mw) were accepted as sound while teeth receiving readings between 1.7 MW and 4 MW were classified as having initial caries. Teeth with readings between 6.00 and 1.50 MW and so on were accepted as having different levels of caries within the tooth.

One operator, skilled in operating of the device performed pre- and post-treatment ECM measurements in accordance with the manufacturer’s instructions. A special in vitro reference electrode which was connected to the root end and in contact with a 0.9% NaCl wetted cotton was used. Before starting ECM measurements, the teeth were wet by 2-3 drops of tap water. With the standard ECM-scale in use (position 2 of the scale selector switch), the operator put the measuring electrode at the center of spot that would be measured. After a short settle time (0.5 seconds), the ECM started the measurement by starting the air-drying with 5 liters/min during 5 seconds. At the end of this period, the measuring electrode was removed and the integrated resistance value in K (kilo) - M (mega) – or G (giga) W (Ohm) was presented in the display. The initial measurements covered all pits and fissures of the occlusal surface. However, two more consecutive measurements were done on the spot with the highest reading. Then, the highest reading obtained on this spot was recorded and its localization was marked on a drawing for future reference.

In Groups 1 and 2, teeth were randomly assigned to 4 subgroups (n= 10). A: Acidulated phosphate fluoride gel (APF); Topex APF Fluoride Gel, Sultan Dental, NJ); B: Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); GC Tooth Mousse, GC Corp., Japan); C: 5% NaF Varnish with...
amorphous calcium phosphate (NaF-ACP); Enamel Pro Varnish, Premier Dental, USA). D: (Control, no treatment).

Table 1 shows the test materials and their ingredients. Three coats of nail varnish was used to completely cover the occlusal surfaces except for the pre-determined spot in the fissures. In the test groups, the topical agents were applied to the spots for 4 minutes. Control group teeth received no treatment. After 4 min, applied materials were cleaned with assigned toothbrushes for 1 minute and washed with distilled water.

The specimens were subjected to pH-cycling. According to pH-cycling model proposed by Featherstone, et al., each subgroup were kept in 40 ml of demineralizing solution (2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer, pH = 4.3) for 6 hours, and in 20 ml of remineralizing solution (1.5 mmol/L calcium, 0.9 mmol/L phosphate, and 150 mmol/L KCl in 0.1 mol/L Tris buffer, pH = 7.0) for 16 hours. Between the demineralizing and remineralizing stages and at the end of pH-cycling, the specimens were washed with deionized and distilled water for 10 seconds and wiped with tissue paper. All solutions were changed daily, and the cycle was repeated for 14 days at 37°C.

After completion of the test procedures ECM readings were repeated. ECM values of pre- and post-treatment of 4 subgroups in Groups 1 and 2 were analyzed by Wilcoxon signed ranks test. Comparison of the two main groups were made by Mann Whitney U test. The level of significance was set as α = 0.05.

RESULTS

The study results were presented in Table 2. In Groups 1A, B and C (incipient fissure caries), pre- and post-treatment ECM readings significantly differed by the application of respective topical agents, APF (p<0.05), CPP-ACP(p<0.01) and NaF-ACP(p<0.05). No significant change was observed in group 1D (control) for pre- and post-treatment ECM readings (p>0.05). With respect to the inter-group differences, statistically significant differences were observed among groups 1 A, B and C (p>0.05, each). However, they were also significantly different than group 1D (p<0.05, each).

In Group 2 where caries-free fissures were treated with the test materials, post-application ECM readings of APF, CPP-ACP and NaF-ACP were found to be significantly different from their respective pre-treatment readings (p<0.01, p<0.01 and p<0.05, respectively). When looked at the inter-group differences, these groups also differed significantly from Group 2 D (p<0.05, each). Group 2 B was significantly different than APF and NaF-ACP (p<0.05), while the differences for the latter groups were insignificant (p>0.05). Finally, the differences between pre- and post application ECM readings in Group 1 and Group 2 were also statistically significant (p<0.01).

DISCUSSION

Due to their complex morphology, occlusal surfaces of teeth are the most susceptible areas for development of caries. Pits and fissures of these surfaces carry more than 85% of the total caries burden while they constitute only 12.5% of total tooth surfaces. Fissure sealants, mainly resin-based materials, are widely used to minimize the caries risk that arises from these sites. However, due to the polymerization shrinkage of resinous materials, it is a well-known fact that microleakage can occur around the sealants applied. The condition may give rise to initiation or progression of carious process which lowers the cost-effectiveness of the procedure.

Therefore, this study aimed to explore the remineralizing potentials of topical agents with different formulations on pits and fissures of occlusal surfaces with or without caries. Acidulated phosphate fluoride (APF) is an effective cariostatic agent which is generally reserved for control of caries in interproximal areas. Maltz et al. carried out a 2-year clinical study in which the effect of an individualized treatment program designed to control occlusal caries in erupting first permanent molars was evaluated. They reported a significant reduction in occlusal caries in children receiving biannual basic preventive program including oral hygiene orientation and toothbrushing with fluoride gel. A similar result has also been reported by Carvalho et al. Under the laboratory conditions of the current study, an anti-caries effect of this material could also be mentioned for both carious and caries-free fissures.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) complex is a milk protein derivative, which has been stated to prevent caries and remineralize enamel. Casein phosphopeptides stabilize and localize ACP in dental plaque, thereby concentrating calcium and phosphate at the tooth surface. By maintaining a high-concentration gradient of calcium and phosphate ions, CPP-ACP helps to suppress demineralization and promote remineralization of enamel by the deposition of apatite. Therefore, its anti-caries effect may be a combination of both. The present study showed that the topical use of CPP-ACP has provided a significant caries-preventive effect in either carious or caries-free fissures.
Based on 5% sodium fluoride (NaF) and amorphous calcium phosphate (ACP), NaF-ACP is a relatively new topical agent in varnish from. Theoretically, it could be expected that, given the same contact time for all test materials, such a varnish could provide better effect when NaF-ACP (Groups 1 C and 2 C) and other test materials were compared. However, with respect to the inter-group differences, only Group 2B significantly differed from other groups in caries-free fissures. The quantification of caries regression in pre-determined carious spots on occlusal surfaces was carried out by ECM. The interaction between the ingredients of NaF-ACP and the carious site might have affected the ECM to accurately quantify the changes in carious fissures. The lack of studies related to the use of this material on fissure caries and the test method used makes comparisons impossible.

In this study, the remineralization effect of test materials were found to be superior on caries-free occlusal surfaces. This finding underlines the importance of providing preventive regimes on erupting and/or newly erupted permanent teeth. Once the carious procedure initiated on an occlusal surface, it would be difficult to reverse (remineralize) the situation due to anatomical and environmental conditions in the oral cavity.

Electrical resistance measurement has been evaluated as a useful technique for fissure caries diagnosis. In a recent study, it has also been used to investigate the posteruptive maturity of fissure enamel. The present study aimed to use this technique to quantify the possible changes in remineralization of occlusal (caries) fissures. It could be stated that the evaluation of remineralization process in these sites might be questionable due to known uncontrollable variables such as debris and humidity. Ekstrand et al. mentioned that there is lack of an accepted clinical gold standard in lesion activity assessment. However, as suggested by Wang et al. ECM could be used as a device to monitor mineral accumulation as a result of preventive treatment in vitro. They have also concluded that it might take a much longer time to follow up the remineralization changes compared with demineralization.

<table>
<thead>
<tr>
<th>Test materials</th>
<th>MATERIAL</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>APF: Topex APF Fluoride Gel (Sultan Dental, NJ, USA)</td>
<td>Sodium fluoride (1.23% fluoride ion), hydrofluoric acid, phosphoric acid, purified water, sodium benzoate, sodium saccharin, xylitol</td>
<td></td>
</tr>
<tr>
<td>CPP-ACP: Tooth Mousse (GC Corp., Tokyo, Japan)</td>
<td>Pure water, glycerol, CPP-ACP, D-sorbitol, silicon dioxide, CMC-Na, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, butyl and propyl p-hydroxybenzoates</td>
<td></td>
</tr>
<tr>
<td>NaF-ACP: Enamel Pro® Varnish (Premier Dental, PA, USA)</td>
<td>5% Sodium fluoride (NaF), amorphous calcium phosphate (ACP)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 1. Test materials</th>
<th>MATERIAL</th>
<th>COMPOSITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>APF: Topex APF Fluoride Gel (Sultan Dental, NJ, USA)</td>
<td>Sodium fluoride (1.23% fluoride ion), hydrofluoric acid, phosphoric acid, purified water, sodium benzoate, sodium saccharin, xylitol</td>
<td></td>
</tr>
<tr>
<td>CPP-ACP: Tooth Mousse (GC Corp., Tokyo, Japan)</td>
<td>Pure water, glycerol, CPP-ACP, D-sorbitol, silicon dioxide, CMC-Na, propylene glycol, titanium dioxide, xylitol, phosphoric acid, guar gum, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, butyl and propyl p-hydroxybenzoates</td>
<td></td>
</tr>
<tr>
<td>NaF-ACP: Enamel Pro® Varnish (Premier Dental, PA, USA)</td>
<td>5% Sodium fluoride (NaF), amorphous calcium phosphate (ACP)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Results obtained in the study (MΩ).</th>
<th>Median</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
<td>Median</td>
<td>Min.</td>
</tr>
<tr>
<td>Group 1 With caries*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1A (APF)*</td>
<td>0.74</td>
<td>0.35</td>
<td>4.23</td>
<td>3.82</td>
</tr>
<tr>
<td>1B (CPP-ACP)*</td>
<td>0.98</td>
<td>0.38</td>
<td>2.81</td>
<td>5.14</td>
</tr>
<tr>
<td>1C (NaF-ACP)*</td>
<td>0.76</td>
<td>0.32</td>
<td>2.93</td>
<td>2.32</td>
</tr>
<tr>
<td>1D (Control)</td>
<td>0.38</td>
<td>0.12</td>
<td>0.96</td>
<td>0.61</td>
</tr>
<tr>
<td>Group 2 Without caries**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A (APF)*</td>
<td>19.11</td>
<td>4.41</td>
<td>42.50</td>
<td>62.95</td>
</tr>
<tr>
<td>2B (CPP-ACP)*</td>
<td>41.42</td>
<td>5.81</td>
<td>63.10</td>
<td>47.90</td>
</tr>
<tr>
<td>2C (NaF-ACP)*</td>
<td>21.40</td>
<td>6.80</td>
<td>86.81</td>
<td>25.95</td>
</tr>
<tr>
<td>2D (Control)</td>
<td>18.85</td>
<td>3.49</td>
<td>25.30</td>
<td>17.50</td>
</tr>
</tbody>
</table>

The statistical significances for inter-group comparisons are shown with different superscripts (a to g, * and **) in rows and cells (p=0.05 and p=0.01).
Instead of using this technique, a smooth surface analysis could be chosen. By using polarized light microscopy, for example, more clear observation of changes in subsurface lesion depths could have been possible. However, technical difficulties in sample preparation from fissures for polarized light microscopy have led us to choose this methodology. Nevertheless, further in vitro and in vivo studies are needed to enlighten this question.

CONCLUSION

Within the limitations of the present study following conclusions could be drawn:
- All test materials showed remineralization effect on both caries free and carious fissures as quantified by ECM.
- CPP-ACP significantly gave better results than APF and NaF-ACP on caries-free occlusal surfaces.
- For all test materials, the remineralization effect was more pronounced on caries-free occlusal surfaces.

ACKNOWLEDGMENT

This study was supported by The Scientific Research and Development Office of Hacettepe University (Project Number: 03-G-021).

REFERENCES


